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# NEURAL CONTROL OF MIGRATION OF PROXIMAL SCREENING PIGMENT BY RETINULAR CELLS OF THE SWIMMING CRAB *CALLINECTES SAPIDUS* [1]

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Arthropods possess compound eyes which, unlike the camera type eye of vertebrates, have a number of fixed aperature lenses. Each lens serves a visual subunit (ommatidium), which is in part comprised of a number of photoreceptors (retinular cells). Each cell contributes part of the photoreceptor organelle (rhabdom) and sends an axon centrally to make synapses with visual interneurons. One of the problems associated with this type of optical system is the control of the amount of flight striking the rhabdom. A general solution to this problem has been the adaptation by which pigment granules are positioned within the ommatidium as a function of the state of light adaptation of the eye. This phenomenon of migration of screening pigment has been the subject of active research for over a century.

These screening pigments can be placed into two broad categories depending upon their position within the ommatidium (Kleinholz, 1966). The first class is located in non-visual accessory cells associated with the ommatidium and includes distal and reflecting type pigments. The second class is located within the retinular cells themselves and is termed proximal screening pigment (PSP). A persistent question has been the control of movement of these pigments; that is, do the cells containing them behave as independent effectors? The answer for the first class appears to be no, since it has been fairly well established that endocrine organs effect the movement of these accessory pigments. The majority of evidence suggests that such influences do not affect movement of pigments within the photoreceptors so that the site of control remains an open question.

Recent ultrastructural observations (Horridge and Barnard, 1965; Eguchi and Waterman, 1967; Kirschfeld and Francheschini, 1969; Butler, 1971; Kolb and Autrum, 1972) suggest that indeed retinular cells do exert direct control over the position of cytoplasmic structures as a function of the state of light adaptation, and in some cases retinomotor phenomena have been shown to cause a change in the gross morphology of the receptor cells (Walcott, 1969, 1971; Debaisieux, 1944). The similarity between these phenomena and the migration of PSP within photoreceptors suggests that the behavior of the PSP could be under neural control of these cells as well. This hypothesis is testable in ommatidia in which (a) PSP does migrate over the axial dimension of the structure and (b) in which one can deliver stimuli which differentially excite photoreceptor elements with that ommatidium.

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The sensitivity of crustacean retinular cells to the plane of polarized light is of particular interest in this regard. Ultrastructural analysis (Eguchi and Waterman, 1966), and microspectrophotometric measurements (Waterman, Fernandez and Goldsmith, 1969) have shown that the basis for this sensitivity lies in the orientation of the microvilli which comprise the rhabdom. Summarily, crustacean ommatidia contain 7 retinular cells, numbered one through seven, over the majority of their length. These cells fall into two classes based on their microvillar orientations. Cell 1 has a microvillar orientation parallel to cells 4 and 5 but occupies a position on the opposite side of the rhabdom. Cells 2 and 3 are adjacent and share a common microvillar axis with cells 6 and 7, also adjacent, located on the opposite side of the rhabdom. Furthermore, the planes of these two microvillar orientations are orthogonal and they align with the vertical axis and horizontal plane of the body. Electrophysiological measurements of polarization sensitivities of individual retinular cells confirm this relation and show that the response of a single cell to a polarized light stimulus oriented parallel to its microvillar axis is often 4 to 6 times greater, in terms of stimulus intensity, than when the same stimulus is oriented orthogonally to that axis, (Waterman and Horch, 1965; Shaw, 1966, 1969; Waterman and Fernandez, 1970; Muller, 1971; Mote, 1972). Therefore, when cells of one class are being most effectively excited, the cells of the other class are receiving stimuli which are severalfold less effective. In this paper we describe experiments in which the position of the PSP in retinular cells of the portunid crab *Callinectes sapidus* was assessed histologically after selective adaptation by polarized light. The results demonstrate that the axial migration of proximal screening pigment is a direct result of receptor excitation and is independent of excitation of other cells in the same ommatidium or eye.

## Materials and Methods

Adult specimens of *Callinectes sapidus* were obtained from food markets in the Philadelphia area shortly after capture by commercial fisheries along the coast of southern New Jersey. They were kept in 100 gallon seawater tanks under constant lighting, at 22 degrees C, and were used within 2 weeks of capture without regard for size or sex. Experiments were performed in two ways. First, intact animals were fixed to a platform, their eye stalks were immobilized with cotton wedges inserted in the orbits. Cotton wicks were placed in contact with the cornea to monitor the ERG (mass response) with standard techniques. The animals were dark adapted for at least an hour following which the evperimental eye was stimulated with flashes of light from a 6V tungsten lamp located 50 cm from the eye. The light was equipped with electromagnetic shutter and polarizing filter (Kodak KN36). The filter was oriented so as to align the e-vector with either vertical axis or horizontal plane of the eye. Flashes of light, 40 msec in duration, were delivered at the rate of 1/sec. The amplitude of the ERG response to these stimuli quickly decreased from its dark adapted value and stabilized some time later at a second value. The flash rate was then decreased until the ERG amplitude was constant at some proportion (25%, 50%, 75%) of the dark adapted value. The eye was flashed at the new rate for 20 minutes. Since the alignment of the plane of polarization of the stimulus and one set of microvillar axes in the eye could

only be approximated in these experiments, a second type of experiment was performed in which proper alignment was assured by monitoring the intracellular response of individual retinular cells to polarized stimuli. Standard microelectrode techniques were employed and are described elsewhere (Bruno, Mote and Goldsmith 1973). In these experiments dark-adapted photoreceptors of isolated eye stalks were exposed by slicing the cornea with a razor blade. The base of the stalk was placed in a seawater bath while the corneal surface was in air and exposed directly to the polarized stimulus source. Maximally and minimally effective polarization orientations for the cell were located by rotating and polarizing filter and observing the response of the cell to flashes of moderate intensity. The eye was then stimulated for 20 minutes with the filter in the maximally effective orientation and the flash rate adjusted so that the amplitude of the cells response to that stimulus stabilized at a new value midway between the dark adapted maximum and minimum. Controls consisted of sham experiments using unpolarized light as the stimulus, and completely light and dark adapted animals.

Immediately following the adaptation treatment eyestalks were placed in a 5% acetic acid solution at 85° C for 30 seconds to halt further pigment movement (L. H. Kleinholz, personal communication). The stalk was then frozen by placing it in a block of dry ice for 60 seconds. The frozen stalk was then bisected in such a way that the stimulated ommatidia remained unaffected by the section. The halves were then placed in 70% ethanol overnight. Following this treatment the retina, optic ganglia and muscles could be teased from the chitinous shell of stalk and cornea, obviating the difficulties of sectioning such materials. The tissue was then inbedded in paraffin, cut into 10 micron sections, lightly stained with haematoxylin and eosin, and mounted in balsam. Photomicrographs were taken under interference contrast microscopy on a Zeiss Ultraphot II light microscope.

## Results

### *Polarization sensitivity*

Retinular cells of *Callinectes* show polarization sensitivity ratios typical of other crustaceans where data is available. These cells respond to light by a depolarization of the resting membrane potential as is common among invertebrates (Fig. 1A). The amount of depolarization is dependent upon the stimulus intensity (Fig. 1B). If plane polarized light is used as a stimulus, then the amount of depolarization is also dependent on the e-vector orientation. Figure 1C shows the modulation in response amplitude as a function of that orientation. The polarizing filter was rotated in 15 degree increments and a 40 msec flash was delivered at each step. The polarization sensitivity ratio can be determined from response-energy relation (Fig. 1B) by locating the maxima and minima of Figure 1C on that curve and measuring the effective intensity of the stimulus at those orientations. The result in this case is a difference equal to about 0.6 log units, or a sensitivity ratio of about 4–1. This value is typical of values of a more extensive study on *Callinectes* (Mote, unpublished).

### *Pigment migration*

Pigments located in non-visual accessory cells (distal and reflecting) in the photopic (apposition) type retina of *Callinectes* appear to be limited to the ex-

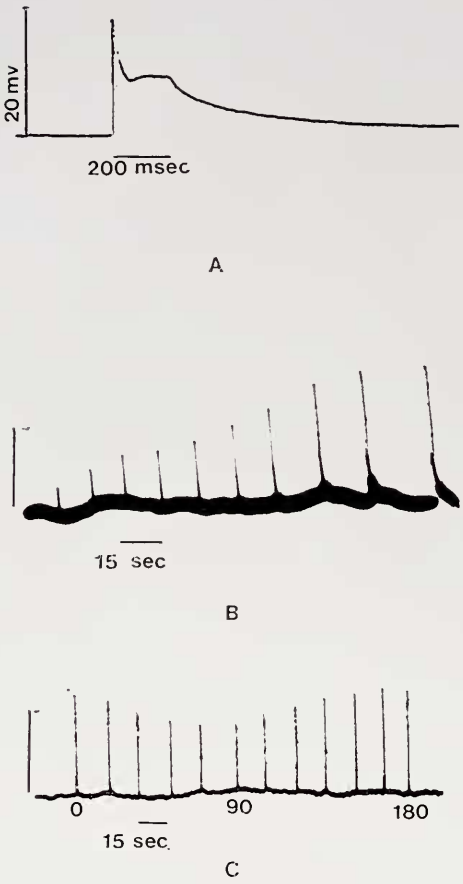


FIGURE 1. Response of a single retinular cell to (a) a 200 msec flash of light of moderate intensity; (b) response of the same cell to a series of 40 msec light flashes of increasing intensity. Each flash is twice (0.3 log units) as bright as the preceding flash and the interval between flashes is 15 seconds; (c) the response of the cell to a series of polarized stimuli of constant intensity and 40 msec duration delivered at an interval of 15 sec. During the interval the polarizing filter was rotated 15°. (The vertical calibration is the same for A, B and C.) The numerals below the trace indicate the cells' response to polarization angles of 90 and 180 degrees.

treme ends of the ommatidium and do not migrate. The distal pigment remains localized in cells around the distal end of the ommatidium where it contacts corneal structures. The reflecting pigment is localized in cells around the proximal end of the ommatidium where the retinular cell axons leave the retina.

The proximal screening pigment can be seen in histological section as a dense globular material restricted to the photoreceptor elements of each ommatidium. It appears to be localized in two masses. An external mass is found in the distal portions of the retinular cells and does not extend more centrally than the outer

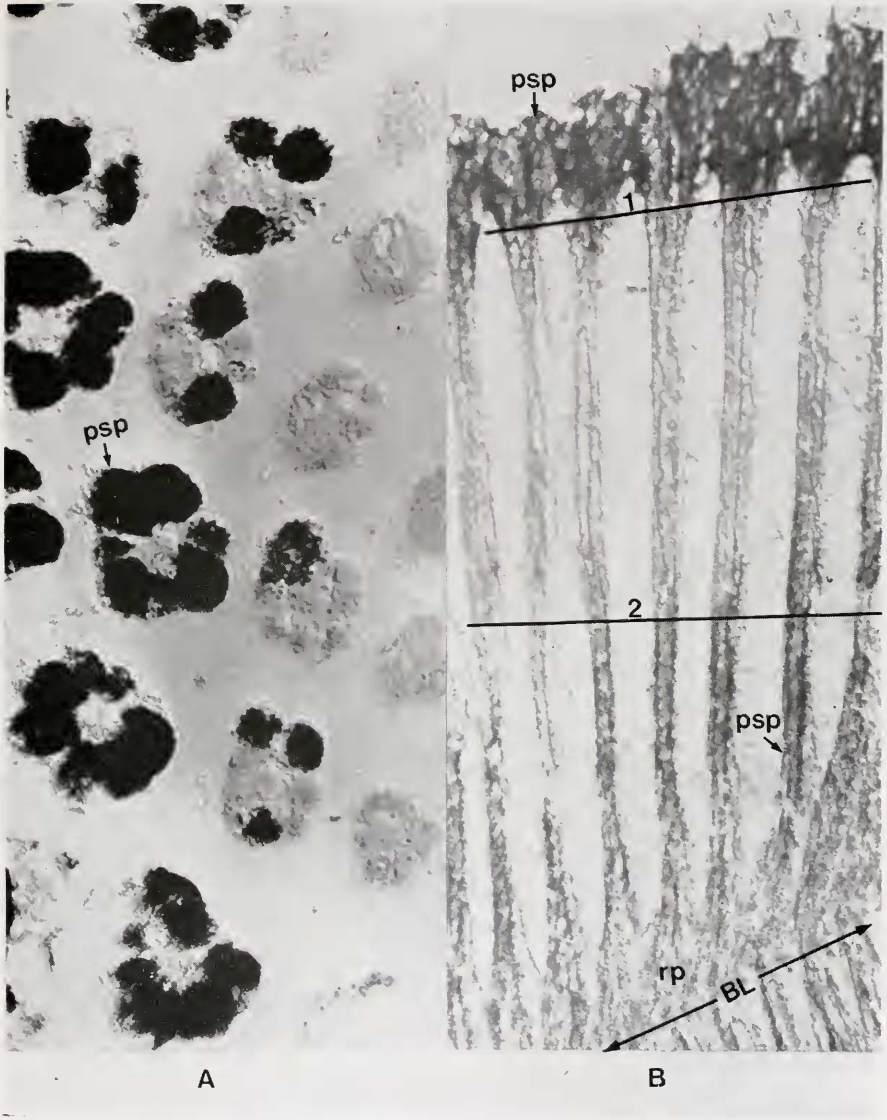


FIGURE 2. Histological section of the retina of *Callinectes sapidus* which has been selectively adapted with polarized light flashes of sufficient intensity and frequency to reduce the amplitude of the ERG response to 50% of its dark adaptd value. The cornea has been removed; (a) an oblique cross section through the retina approximately along the line marked 1 in 2B; (b) longitudinal section of several adjacent ommatidia. The thickened distal ends (top) contain the dense proximal screening pigment (PSP) which extends centrally to the region marked by line 1. The central regions of the cell also contain this material which extends distally to the area marked by line 2. Reflecting pigment (rp) is found on both sides of the basement lamina (BL) which borders the receptor layer centrally.

20% of the ommatidium (Fig. 2B). There is some suggestion ot movement of this pigment mass, but if it occurs it is so restricted that it is very difficult to assess (Fig. 2A and discussion). An internal mass of the PSP is located in the proximal regions of the retinular cell and appears to migrate extensively (Fig. 2B). In animals that have been strongly light-adapted these two masses meet and the cell is completely filled with the dense screening material. In moderately light-adapted animals there is a gap between the two masses as shown in Figure 2B, and in dark-adapted animals the internal mass migrates centrally to the level of the basement lamina (Fig. 2B) and is not seen peripherally.

Longitudinal sections are useful in determining the position of the PSP within the retina, however they do not allow determination of the position of the pigment within the elements of a single ommatidium. To determine the relative distribution of pigment within the receptor cells of a single ommatidium, the ommatidium was examined in serial cross sections. Figure 3 is a photomicrograph of a cross section (approximately at the level indicated by line 2 in Fig. 2B) through a retina adapted by polarized light whose e-vector had been oriented by monitoring the intracellular response. It consists of adjacent ommatidia representing elements from three adjacent rows in the two axes of the eye and demonstrates that the PSP is not evenly distributed through all cells of each ommatidium. Furthermore it demonstrates (1) that this lack of symmetry is consistent in form among neighboring elements, (2) that it aligns with a row marking the horizontal axis of the eye (large arrow at center), and (3) that the pigment can be found on opposite sides of the rhabdom (small arrows). The form of the asymmetry is not restricted to a particular section but is also consistent along the axial dimension of the ommatidium. Figure 4 represents a series of cross sections of a single ommatidium over about 100 micra of its 300 micron length. It was taken from the same retina as that shown in Figure 3. The sections proceed proximally in 10 micron steps except where sections omitted because of redundancy (see caption). The PSP first appears along the rhabdom as a thin layer which extends for about 50 microns (Fig. 4A–D), at which point it appears around 2 adjacent reticular cell nuclei and on the opposite side of the rhabdom (Fig. 4E). In Figure 4F the pigment has surrounded the rhabdom but is predominantly located in the elements on opposite sides, and finally (Fig. 4E–I) is distributed equally in all elements. Figure 5 represents a series taken from a retina treated identically to that depicted in Figures 3 and 4 but omits alternate sections. It demonstrates the essential features [*i.e.*, (a) appearance of pigment first along the rhabdom, and (b) then around the nucleus, (c) in cells on opposite sides of the rhabdom, and (d) finally in all elements] that are similar in all serial sections we were able to construct. Thus it appears that the PSP can be localized in different axial positions in different retinular cells of a given ommatidium that has been selectively adapted with polarized light. Furthermore this distribution appears to be consistent among neighboring ommatidia in a given region of the eye. In 15 selective adaptation experiments, results of histological examination suggested uneven pigment distribution while in 5 sham control experiments, or in fully light or dark adapted eyes they did not.

In experiments on intact eyes, the orientation of the adapting filter with respect to the major axes of the eye was only approximated and the process of

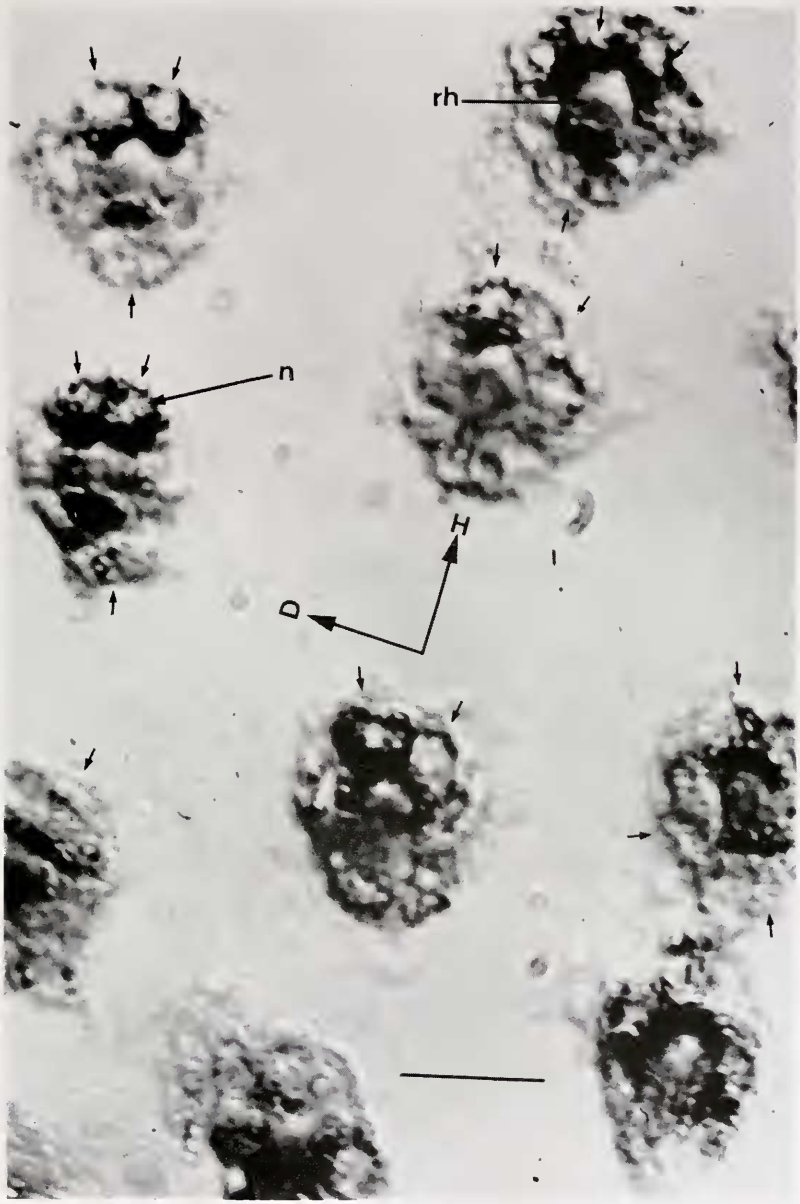


FIGURE 3. Cross section through a retina selectively adapted with flashes of polarized light whose e-vector orientation had been determined by monitoring the intracellularly recorded receptor potential. The plane of section coincides approximately with line 2 of Figure 2B. The proximal screening pigment appears in various elements of each ommatidium and is marked by the small arrows. The large arrows note the dorsoventral (D) axis and horizontal (H) axis of the eye. Each ommatidium shows a pigment free central area, the rhabdom (rh), and pigment free peripheral areas, the retinular cell nuclei (n). Scale bar indicates 20 microns.

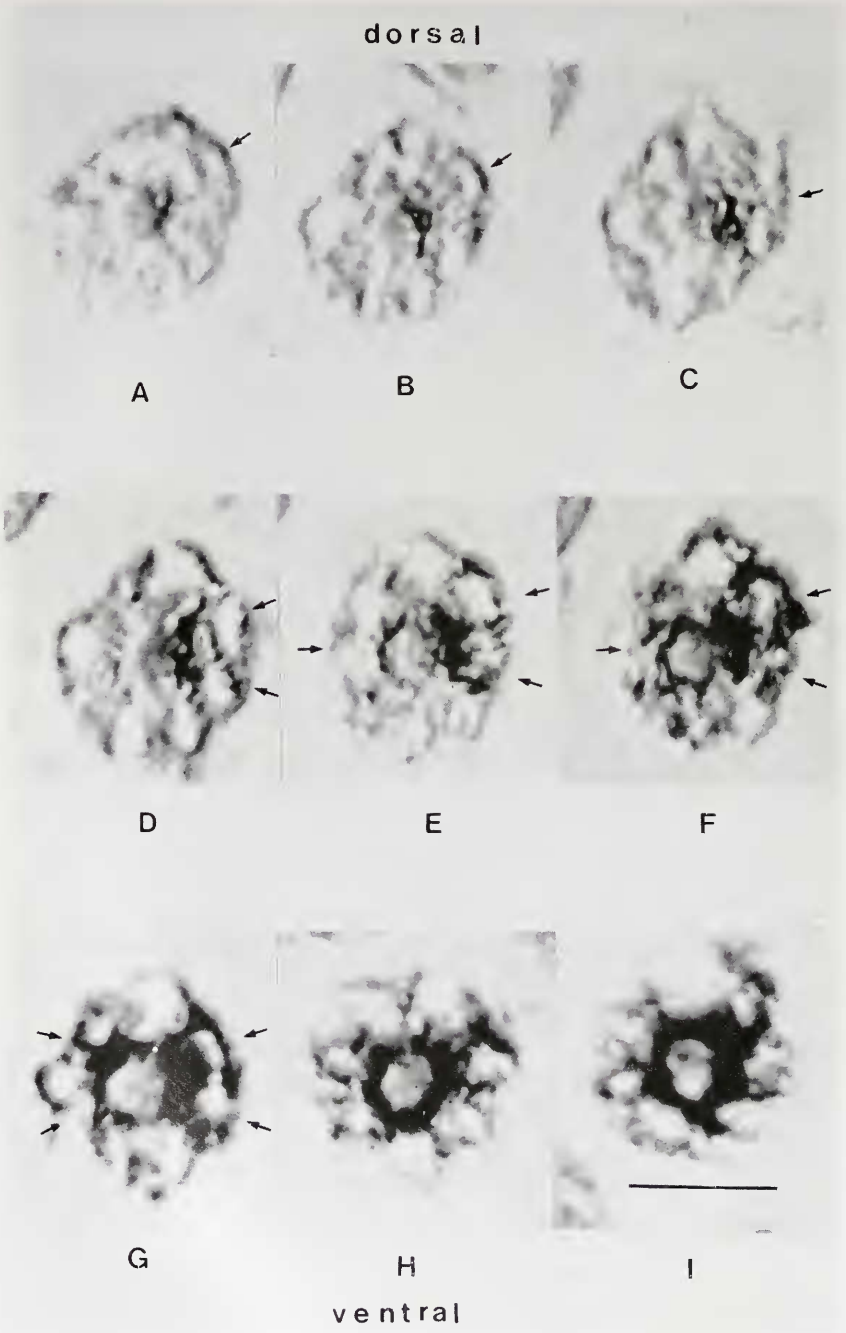


FIGURE 4. Serial cross sections through a single ommatidium from a retina selectively adapted with flashes of polarized light whose e-vector had been oriented by monitoring the intracellular response. The first section (A) was made approximately 100 microns from the

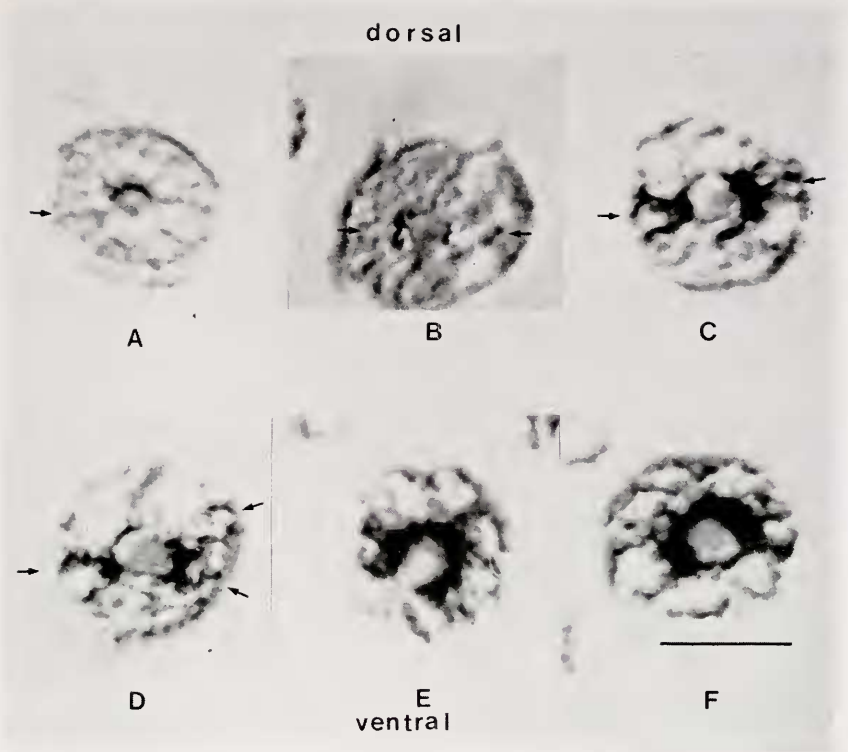


FIGURE 5. Serial cross sections through an ommatidium in a retina treated as in Figure 4. The first section (A) begins at about the same depth as in Figure 4, but only alternate sections are shown. Thus the axial extent of the series is about 100 microns. Small arrows in sections B–D indicate where the proximal pigment can be observed. Scale bar indicates 20 microns.

preparation for histological section produced major uncertainties regarding the orientation of tissue in the paraffin block. We can, however, estimate the relation of pigment position to the major axes in experiments where the filter was positioned by monitoring the intracellular response to polarized stimuli because in this type of experiment the retina was sliced in a plane which was approximately parallel to the horizontal axis, the cut edge then providing a landmark in each section. These estimations are included in Figures 3, 4 and 5. It must be noted that in such experiments the filter position was appropriate for a single cell and its relation to the whole eye can only be inferred. Furthermore, we cannot preclude distortions caused by electrode impalement or histological preparation.

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distal end of the ommatidium (roughly midway between lines 1 and 2 of Fig. 2B) and proceed centrally at 10 micron intervals. One section has been omitted between C and D, and between G and H so that the axial extent of the series is about 110 microns. Small arrows in sections A–C indicate regions of the ommatidium where the proximal screening pigment can be observed. In sections H and I the pigment clearly surrounds the entire rhabdom. Scale bar indicates 20 microns.

Given these reservations, we feel that the ommatidial diameter along which the PSP is distributed aligns reasonably well with the estimated horizontal axis of the eye stalk in Figures 3, 4 and 5.

## Discussion

The only motile pigment found in the retina of *Callinectes sapidus* was that which was localized in the photoreceptor elements and can be classified as proximal screening pigment (PSP). Our examinations revealed that it is found in two masses, external and internal. The external mass, if mobile at all, remains in the peripheral regions of the ommatidium. Sections through this region of the retina occasionally suggested uneven distribution of this material (Fig. 2A), however, the restricted region of the ommatidium in which this material could be observed makes it difficult to eliminate the possibility of artifact or make any clear statement about its mobile properties. The internal mass of PSP, on the other hand, can clearly be found in different positions within the retinular cells of the same ommatidium when the retina had been adapted with polarized light. Intracellular measurements of polarization sensitivity ratios in these cells yielded values of between 4 and 6 to 1. This is the same as saying that a given polarized stimulus, if properly oriented, excites one set of receptors (*e.g.*, 2, 3, 6 and 7) as though it were a light 4 to 6 times brighter (or dimmer) than the other set (*e.g.*, 1, 4 and 5). If the screening pigment were to move independently in each set as a function of the stimulus intensity, then one would expect to find the pigment in different positions in each set. Our results suggest that this is the case.

The difficulties inherent in properly orienting and sectioning experimental eyes raise the possibility that such results are artifactual. The ultrastructure of the ommatidium as described above poses certain restrictions upon the types of uneven distributions one would expect to find if the pigment movement were in response to the adapting stimulus. First, the distribution should be similar in a number of adjacent ommatidia since their microvillar axes are in register along each row and a stimulus properly aligned for one would be properly aligned for others as well. Figure 3 demonstrates that this requirement is fulfilled. The elements depicted there show pigment distributions comparable to each other and these distributions align with the axis of the row. Secondly, since retinular cells on opposite sides of the rhabdom are receiving stimuli which are equally effective (see above) one would expect the pigment to appear along a diameter of the ommatidium. Figures 3, 4 and 5 demonstrate that this requirement is fulfilled. We imposed the further requirement that we be able to follow a single ommatidium in serial sections and observe the appearance of the PSP at its distal limit. This is a severe requirement for several reasons. First, the ommatidial axes are not parallel so that the chance that a given plane of section will cut the same element in cross section over a hundred microns is small. Secondly, the theoretical treatment of Snyder and Pask (1972), based on the rhabdom of the honey bee, suggests that the polarization sensitivity will fall off sharply as the position of the stimulus deviates from the optical axis of the ommatidium. This means that in our experiments a relatively small number of ommatidia would be receiving the appropriate stimulus from a localized source 50 cm away, and the difficulty of obtaining them in cross section is compounded.

We feel that the serial sections depicted in Figures 4 and 5 fill this requirement. They demonstrate that the observed distributions persist over some distance and that the transition from asymmetrical to symmetrical distribution is gradual. We consider it unlikely that an artifact created by the plane of section or tissue damage during sectioning, could result in such observations. This, coupled with the agreement between our results and the predictions from ultrastructural analysis and electrophysiology, lead us to conclude that the hypothesis that these photoreceptors exert direct action on the pigment material within them is correct. Unfortunately our experiments do not enable us to comment on the mechanism of movement or even quantitatively describe it.

One must use caution, however, in generalizing these results to other species. Kleinholz (1961) points out that the presence of the different types of screening pigments and their motility is variable in different species. On the other hand, the preponderance of evidence supports hormonal control of distal and reflecting pigment migration where they have been observed, while such a mechanism does not appear to affect migration of the PSP. In this light, our experiments suggesting neural control of PSP migration tempt the speculation that such a mechanism is a general property of arthropod photoreceptor cells. In addition, these experiments and their results serve as further evidence of the functional independence of retinular cells within each ommatidium of the arthropod compound eye even though these cells share a common rhabdom.

We wish to thank Professor Lewis Kleinholz for his helpful advice on histological techniques.

## Summary

1. The position of proximal screening pigment (PSP) located in retinular cells of the compound eye of the crab *Callinectes sapidus* was assessed histologically after selective adaptation with plane polarized light.

2. The results showed the pigment position to be different within the cells of a single ommatidium.

3. The pigment position was similar in cells of that ommatidium which shared a common microvillar axis and different from those that did not.

4. The pattern of pigment distribution was similar in several adjacent ommatidia.

5. Serial sections showed that the pigment in cells sharing a common microvillar axis extended more distally than those that did not after a polarized adapting stimulus.

6. These results support the hypothesis that the migration of PSP is under neural control of the retinular cell where it is found and independent of activity in other cells in the same ommatidium or eye.

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